FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
12020-0003	09/719088

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU99/00523	29 June 1999 (29.06.99)	29 June 1998 (29.06.98)

TITLE OF INVENTION
NPY-Y7 Receptor Gene

APPLICANT(S) FOR DO/EO/US
Herbert HERZOG

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☐ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Sequence Listing Material (disk and paper copy)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/719088	PCT/AU99/00523	12020-0003

21. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☒	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,000.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO **$860.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**	
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$1,000.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	25 - 20 =	5	x $18.00	**$90.00**
Independent claims	3 - 3 =	0	x $80.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**
	TOTAL OF ABOVE CALCULATIONS		=	**$1,090.00**
	Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.		☐	**$0.00**
	SUBTOTAL		=	**$1,090.00**
	Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).		+	**$0.00**
	TOTAL NATIONAL FEE		=	**$1,090.00**
	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**		☒	**$40.00**
	TOTAL FEES ENCLOSED		=	**$1,130.00**
			Amount to be: refunded	$
			charged	$

☒ A check in the amount of **$1,130.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **50-1088** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Christopher W. Brody
SIGNATURE

Christopher W. Brody
NAME

33,613
REGISTRATION NUMBER

December 8, 2000
DATE

#8
8/B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
U.S. Designated/Elected Office (DO/EO/US)

In re Application of:

HERZOG

Int'l Application No. PCT/AU99/00523

Int'l Filing Date: 29 June 1999 (29.06.99)

For: NPY-Y7 Receptor Gene

SUPPLEMENTAL PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to calculating the official fees in the above-captioned application, please amend the application as follows:

IN THE CLAIMS:

Claim 11 (twice amended) A host cell transformed with a polynucleotide molecule encoding an NPY-Y7 receptor or a functionally equivalent fragment thereof, wherein the encoded NPY-Y7 receptor is characterized by the N-terminal amino acid sequence:

$MX_1X_2MX_3EKWDX_4NSSE$ (SEQ ID NO: 1),

wherein X_1, X_2, X_3, and X_4 are selected from codable amino acids or a plasmid or expression vector according to claim 10.

Claim 22 (twice amended) A method for detecting agonist or antagonist agents of an NPY-Y7 receptor, comprising contacting an NPY-Y7 receptor which is characterized by the N-terminal amino acid sequence:

$MX_1X_2MX_3EKWDX_4NSSE$ (SEQ ID NO:1),

wherein X_1, X_2, X_3 and X_4 are selected from codable amino acids, or a functionally equivalent fragment of said receptor, in a substantially pure form or a host cell transformed according to claim 14, with a test agent under conditions enabling the activation of said receptor, and detecting an increase or decrease in the receptor activity.

REMARKS

The above amendments are made to delete multiple dependency in the claims. No new matter is contained in the amendment.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 50-1088.

Respectfully submitted,

CLARK & BRODY

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Docket No.: 12020-0003
Date: December 8, 2000

MARKED-UP CLAIMS

Claim 11 (once amended) A host cell transformed with a polynucleotide molecule [according to] encoding an NPY-Y7 receptor or a functionally equivalent fragment thereof, wherein the encoded NPY-Y7 receptor is characterized by the N-terminal amino acid sequence:

$MX_1X_2MX_3EKWDX_4NSSE$ (SEQ ID NO: 1),

wherein X_1, X_2, X_3, and X_4 are selected from codable amino acids [any one of claims to 9] or a plasmid or expression vector according to claim 10.

Claim 22 (once amended) A method for detecting agonist or antagonist agents of an NPY-Y7 receptor, comprising contacting an NPY-Y7 receptor [according to] which is characterized by the N-terminal amino acid sequence:

$MX_1X_2MX_3EKWDX_4NSSE$ (SEQ ID NO:1),

wherein X_1, X_2, X_3 and X_4 are selected from codable amino acids, or a functionally equivalent fragment of said receptor, in a substantially pure form [any one of claims 15-19] or a host cell transformed according to claim 14 [any one of claims 11 to 14], with a test agent under conditions enabling the activation of said receptor, and detecting an increase or decrease in the receptor activity.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
U.S. Designated/Elected Office (DO/EO/US)

In re Application of:

HERZOG

Int'l Application No. PCT/AU99/00523

Int'l Filing Date: 29 June 1999 (29.06.99)

For: NPY-Y7 Receptor Gene

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Prior to calculating the official fees in the above-captioned application, please amend the application as follows:

IN THE CLAIMS:

Please amend claims 3, 5, 9, 10, 11, 14, 20, 21, 22, 23, 24 and 25 as follows:

In claim 3, line 1, please delete "or 2".

In claim 5, line 1, please delete "or 2".

In claim 9, line 1, please delete "or 8".

In claim 10, line 2, please change "any one of claims 1 to 9", to --claim 1--.

Claim 11. (once amended) A host cell transformed with a polynucleotide molecule according to claim 1 [any one of claims to 9] or a plasmid or expression vector according to claim 10.

In claim 14, line 1, please change "any one of claims 11 to 13", to --claim 11--.

In claim 20, line 2, please change "any one of claims 15 to 19", to --claim 15--.

Claim 21. (once amended) A non-human animal transformed with [a polynucleotide molecule according to claim 1 [to any one of claims 1 to 9 or] a plasmid or expression vector according to claim 10.

Claim 22 (once amended) A method for detecting agonist or antagonist agents of an NPY-Y7 receptor, comprising contacting an NPY-Y7 receptor according to claim 15 [any one of claims 15-19] or a host cell transformed according to claim 14 [any one of claims 11 to 14], with a test agent under conditions enabling the activation of said receptor, and detecting an increase or decrease in the receptor activity.--

In claim 23, line 4, please change "any one of claims 1 to 9", to --claim 1--.

In claim 24, line 4, please change "any one of claims 1 to 9", to --claim 1--.

Claim 25 (once amended) A method of producing NPY-Y7 receptors or functionally equivalent fragments thereof, the receptor characterized by the N-terminal amino acid sequence:

$MX_1X_2MX_3EKWDX_4NSSE$ (SEQ ID NO:1)

Wherein X_1, X_2, X_3, AND X_4 are selected from codable amino acids, or a functionally equivalent fragment of said receptor, in a substantially pure form [according to any one of claims 15 to 19], comprising culturing a host cell according to claim 14 [any one of claims 11-14] under conditions enabling the expression of NPY-Y7 receptors or functionally equivalent fragments thereof, and optionally recovering the receptors or functionally equivalent fragments thereof. .--

REMARKS

The above amendments are made to delete multiple dependency in the claims. No new matter is contained in the amendment.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 50-1088.

Respectfully submitted,

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Date: December 8, 2000

NPY-Y7 RECEPTOR GENE

Field of Invention:

The present invention relates to isolated polynucleotide molecules which encode a novel neuropeptide Y (NPY) receptor designated NPY-Y7. In addition, the present invention relates to the use of these molecules in the production of NPY-Y7 receptors using recombinant DNA technology and to methods of screening and testing compounds for agonist or antagonist activity.

Background of the Invention:

Neuropeptide Y (NPY) forms a family (called the pancreatic polypeptide family) together with pancreatic polypeptide (PP) and peptide YY (PYY), which all consist of 36 amino acids and possess a common tertiary structure. NPY receptors, members of the G protein- coupled receptor superfamily, when activated influence a diverse range of important physiological parameters, including effects on psychomotor activity, central endocrine secretion, anxiety, reproduction, vasoactive effects on the cardiovascular system and strongly stimulates food consumption. Specific agonists and antagonists of NPY are therefore likely to be of substantial benefit for therapy of a wide range of clinical disorders. As NPY possess a compact tertiary structure and different parts of the molecule are required for interaction with different subtypes of the receptor, the logical developments of both agonists and antagonists is critically dependent upon the availability and knowledge of specific receptor structure.

It is presently known that NPY binds specifically to at least six receptors; Y1, Y2, Y3, Y4, Y5 (or "atypical Y1") and Y6. While it has been demonstrated that NPY receptors couple to the adenylate cyclase second messenger system, it remains probable that additional NPY receptor subtypes exist since there is evidence that phosphatidylinositol turnover, cations, and arachidonic acid may also function as second messengers for NPY.

Since NPY agonists and antagonists may have commercial value as, for example, potential anti-hypertensive agents, cardiovascular drugs, neuronal growth factors, anti-psychotics, anti-obesity and anti-diabetic agents, the ability to produce NPY receptors by recombinant DNA technology would be

advantageous. To this end, DNA molecules encoding Y1, Y2, Y4, Y5 and Y6 have previously been isolated.

The present inventors have now isolated novel DNA molecules encoding the human and murine NPY-Y7 receptors.

Summary of the Invention:

Thus, in a first aspect, the present invention provides an isolated polynucleotide molecule encoding an NPY-Y7 receptor or a functionally equivalent fragment thereof.

The encoded NPY-Y7 receptor is characterised by the N-terminal amino acid sequence:

$MX_1X_2MX_3EKWDX_4NSSE$ (SEQ ID NO: 1),

wherein X_1, X_2, X_3 and X_4 are selected from codable amino acids but, preferably, X_1 is selected from Phe and Ser, X_2 is selected from Ile and Thr, X_3 is selected from Asn and Ser, and X_4 is selected from Thr and Ser.

More preferably, the polynucleotide molecule encodes a human NPY-Y7 receptor of about 408 amino acids or a murine NPY-Y7 receptor of about 405 amino acids.

Most preferably, the polynucleotide molecule encodes a human NPY-Y7 receptor having an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2 or a murine NPY-Y7 receptor having an amino acid sequence subtantially corresponding to that shown as SEQ ID NO: 3.

The polynucleotide molecule may comprise a nucleotide sequence substantially corresponding or, at least, showing at least 90% (more preferably, at least 95%) homology to that shown at nucleotides 1 to 1903 or nucleotides 369 to 1592 of SEQ ID NO: 4 or any portion thereof encoding a functionally equivalent NPY-Y7 receptor fragment.

The polynucleotide molecule may be incorporated into plasmids or expression vectors (including viral vectors), which may then be introduced into suitable bacterial, yeast, insect and mammalian host cells. Such host cells may be used to express the NPY-Y7 receptor.

Accordingly, in a second aspect, the present invention provides a mammalian, insect, yeast or bacterial host cell transformed with the polynucleotide molecule of the first aspect.

In a third aspect, the present invention provides a method of producing NPY-Y7 receptors or functionally equivalent fragments thereof, comprising

culturing the host cell of the second aspect under conditions enabling the expression of NPY-Y7 receptors or functionally equivalent fragments thereof.

Preferably, the host cell is mammalian or of insect origin. Where the cell is mammalian, it is presently preferred that it be a Chinese hamster ovary (CHO) cell, monkey kidney (COS) cell or human embryonic kidney 293 cell. Where the cell is of insect origin, it is presently preferred that it be an insect Sf9 cell.

In a preferred embodiment, the NPY-Y7 receptors or functionally equivalent fragments thereof are expressed onto the surface of the host cell.

The polynucleotide molecule of the present invention encodes an NPY receptor which may be of interest both clinically and commercially as it is expressed in many regions of the body and neuropeptides of the NPY family affect a wide number of systems.

By using the polynucleotide molecule of the present invention it is possible to obtain NPY-Y7 receptor protein or fragments thereof in a substantially pure form.

Accordingly, in a fourth aspect, the present invention provides a NPY-Y7 receptor or a functionally equivalent fragment of said receptor, in a substantially pure form.

In a fifth aspect, the present invention provides an antibody or fragment thereof capable of specifically binding to the NPY-Y7 receptor or functionally equivalent fragment of the fourth aspect.

In a sixth aspect, the present invention provides a non-human animal transformed with the polynucleotide molecule of the first aspect of the present invention.

In a seventh aspect, the present invention provides a method for detecting agonist or antagonist agents of an NPY-Y7 receptor, comprising contacting an NPY-Y7 receptor, functionally equivalent fragment thereof or a cell transfected with and expressing the polynucleotide molecule of the first aspect, with a test agent under conditions enabling the activation of an NPY-Y7 receptor, and detecting an increase or decrease in activity of the NPY-Y7 receptor or functionally equivalent fragment thereof.

An increase or decrease in activity of the receptor or functionally equivalent fragment thereof may be detected by measuring changes in cAMP production, Ca^{2+} levels or IP3 turnover after activating the receptor or fragment with specific agonist or antagonist agents.

In a further aspect, the present invention provides an oligonucleotide or polynucleotide probe comprising a nucleotide sequence of 10 or more nucleotides, the probe comprising a nucleotide sequence such that the probe specifically hybridises to the polynucleotide molecule of the first aspect under high stringency conditions (Sambrook et al., *Molecular Cloning: a laboratory manual*, Second Edition, Cold Spring Harbor Laboratory Press).

In a still further aspect, the present invention provides an antisense oligonucleotide or polynucleotide molecule comprising a nucleotide sequence capable of specifically hybridising to an mRNA molecule which encodes an NPY-Y7 receptor so as to prevent translation of the mRNA molecule.

Such antisense oligonucleotide or polynucleotide molecules may include a ribozyme region to catalytically inactivate mRNA to which it is hybridised.

The polynucleotide molecule of the first aspect of the invention may be a dominant negative mutant which encodes a gene product causing an altered phenotype by, for example, reducing or eliminating the activity of endogenous NPY-Y7 receptors.

The term "substantially corresponding" as used herein in relation to amino acid sequences is intended to encompass minor variations in the amino acid sequences which do not result in a decrease in biological activity of the NPY-Y7 receptor. These variations may include conservative amino acid substitutions. The substitutions envisaged are:-

G, A, V, I, L, M; D, E; N, Q; S, T; K, R, H; F, Y, W, H; and P, Nα-alkalamino acids.

The term "substantially corresponding" as used herein in relation to nucleotide sequences is intended to encompass minor variations in the nucleotide sequences which due to degeneracy in the DNA code do not result in a change in the encoded protein. Further, this term is intended to encompass other minor variations in the sequence which may be required to enhance expression in a particular system but in which the variations do not result in a decrease in biological activity of the encoded protein.

The term "functionally equivalent fragment/s" as used herein is intended to refer to fragments of the NPY-Y7 receptor that exhibit binding specificity and activity that is substantially equivalent to the NPY-Y7 receptor from which it/they is/are derived.

The terms "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated step, component or feature or group of steps, components or features with or without the inclusion of a further step, component or feature or group of steps, components or features.

Reference to percent homology made in this specification have been calculated using the BLAST program blastn as described by Altschul, S.F. et al., "Capped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Research*, Vol. 25, No. 17, pp. 3389-3402 (1997).

Brief description of the accompanying Figures:

Figure 1 shows the degree of identity between the predicted amino acid sequence of the human NPY-Y1, NPY-Y2 and NPY-Y7 receptors.

Figure 2 provides a graph showing the inhibition of human [^{125}I]PYY binding with various NPY-related peptides on human NPY-Y7 membranes. The results were obtained through competitive displacement of [^{125}I]PYY on membranes of COSm6 cells transiently expressing human NPY-Y7 receptors. Membranes were incubated with [^{125}I]PYY (50pM) and increasing concentrations of peptide competitors. Data are representative of a single experiment with each point measured in triplicate.

Figure 3 provides a schematic diagram of the murine NPY-Y7 receptor gene. The gene covers approximately 12 kb and consists of three exons.

Figure 4 shows the degree of identity between the predicted amino acid sequence of the human and murine NPY-Y7 receptors.

Detailed Disclosure of the Invention:

Human NPY-Y7 cDNA

Human amygdala and testis cDNA libraries (Stratagene) were screened under low strigency conditions with a 401 bp ^{32}P-labelled fragment (corresponding to nucleotides 507 to 908 of SEQ ID NO: 4) originated from a human fetal brain EST clone (GenBank AA449919). Two overlapping cDNA clones were obtained from the screen. The combined nucleotide sequence (hy7) of the clones is shown as SEQ ID NO: 4 and encodes a protein of 408 amino acids (SEQ ID NO: 2).

Sequence comparison with other G protein coupled receptors identified neuropeptide Y receptors as the most closely related group with approximately 32% amino acid sequence identity to the Y1 receptor subtype (Figure 1). Further, *in situ* hybridisation studies of rat brain sections has identified a NPY-Y7 mRNA distribution (expression was found to occur in the amygdala, the CA3 region of the hippocampus and the piriform cortex) which is consistent with the expression of other NPY-receptor subtypes (Blomquist, A.G., and Herzog, H., TINS 20(7), 1997) and is in agreement with the suggestions of the existence of further Y-receptor family members. This mRNA distribution suggests important functions for the NPY-Y7 receptor in the regulation of the circadian rhythm, anxiety and metabolic status.

Radio-ligand binding experiments has shown that the protein encoded by the hy7 cDNA shows highest affinity for human PYY (Figure 2). These experiments were conducted using COS-6 or HEK (293) cells transiently expressing recombinant Y7 receptor protein. The radio-ligand binding (Herzog, H. et al., Proc. Natl. Acad. Sci. USA 89:5794-5798, 1992) suggests that the NPY-Y7 receptor has a pharmacology similar to the Y2 receptor (Rose, P., J. Biol. Chem. 270:22661-22664, 1995). The rank of potency for the Y7 receptor is:

PYY>NPY>[2-36]PYY>[3-36]NPY>[13-36]NPY>>(Leu31, Pro34)NPY>PP.

Chromosomal Localisation of the Human Y7 gene

Screening of a medium resolution Stanford G3 panel of 83 clones was performed to further refine the map position of the hy7 gene. PCR amplification was carried out on this panel using primers hy7-A (5'GGATGGCCATTTGGAAAC3') and hy7-B (5'CCAATCCTTCCATACATG3'), corresponding to nucleotides 507-524 and 890-907 of the hy7 cDNA (SEQ ID NO: 4), respectively. The analysis indicated that the hy7 gene is most closely associated with the marker SHGC-418 on the long arm of chromosome 4. This map location is defined by markers AFM191xh2 and AFM347ZH1. Assessment of the flanking markers using the Whitehead/MIT STS-Based Map of the Human Genome)(http://www-genome.wi.mit.edu/cgi-bin/contig/phys_map) in conjunction with The Genome Directory (Adams, M.D., et al. Nature 377 Suppl. (1995)) identifies 4q21.3 as the most likely position of the hy7 gene.

Mouse Y7 genomic DNA

Using a ^{32}P-labelled fragment of the hy7 cDNA a mouse genomic BAC library (Genome Systems) was screened. A clone encoding the entire gene of the mouse equivalent to hy7 was isolated (SEQ ID NO: 5). The gene covers approximately 12 kb and is divided by two introns into three exons (Figure 3). Figure 4 shows the degree of identity between the predicted amino acid sequence of the human and murine NPY-Y7 receptors.

Pharmacological characterisation

pcDNA3.1-hy7 cDNA was transiently transfected into the COSm6 cell line using FUGENE and 5mg of DNA/106 cells. The COSm6 cells were grown in Dulbecco's modified Eagles medium supplemented with 2mM glutamine and 10% fetal calf serum, in 5% CO_2 at 37°C. Membranes were harvested with COSm6 cells 72hr post-transfection. Adherent cells were washed twice in ice-cold phosphate buffered saline and lysed using a glass homogeniser in ice-cold hypotonic buffer (50mM Tris-HCl, pH 7.4, 0.1% bacitracin). Membranes were pelleted by high speed centrifugation (30,000 x g, 15min, 4°C), homogenised again in ice-cold hypotonic buffer and collected again by high speed centrifugation (30,000 x g, 15min. 4°C). The final membrane pellet was resuspended into 1ml of ice-cold binding buffer (50mM Tris-HCl, pH7.4, 10mM NaCl, 5mM MgCl2, 2.5mM CaCl2, 0.1% bacitracin, 0.1% bovine serum albumin. Membrane suspensions were diluted in binding buffer to yield membrane protein concentrations of 0.05mg/ml. Under these conditions non-specific binding of [^{125}I]PYY to membranes was less than 10%. [^{125}I]PYY and unlabelled peptide competitors were also diluted to the required concentrations in binding buffer. Samples were prepared by mixing 50ml binding buffer, unlabelled peptide or binding buffer (50ml), [^{125}I]PYY (50mM, 50ml) and membrane suspension (100ml). Samples were incubated at room temperature for 2hr. Incubations were terminated by centrifugation (4min) and pellets collected. Radioactivity was measured for 1min in a g counter.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Claims:

1. An isolated polynucleotide molecule encoding an NPY-Y7 receptor or a functionally equivalent fragment thereof, wherein the encoded NPY-Y7 receptor is characterised by the N-terminal amino acid sequence:

$MX_1X_2MX_3EKWDX_4NSSE$ (SEQ ID NO: 1),

wherein X_1, X_2, X_3 and X_4 are selected from codable amino acids.

2. A polynucleotide molecule according to claim 1, wherein X_1 is selected from Phe and Ser, X_2 is selected from Ile and Thr, X_3 is selected from Asn and Ser and X_4 is selected from Thr and Ser.

3. A polynucleotide molecule according to claim 1 or 2, wherein the polynucleotide molecule encodes an NPY-Y7 receptor of human origin of about 408 amino acids in length.

4. A polynucleotide molecule according to claim 3, wherein the polynucleotide molecule encodes a human NPY-Y7 receptor having an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

5. A polynucleotide molecule according to claim 1 or 2, wherein the polynucleotide molecule encodes an NPY-Y7 receptor of murine origin of about 405 amino acids in length.

6. A polynucleotide molecule according to claim 5, wherein the polynucleotide molecule encodes a murine NPY-Y7 receptor having an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 3.

7. A polynucleotide molecule encoding an NPY-Y7 receptor, wherein the polynucleotide molecule comprises a nucleotide sequence showing at least 90% homology to that shown at nucleotides 1 to 1903 or nucleotides 369 to 1592 of SEQ ID NO: 4 or any portion thereof encoding a functionally equivalent NPY-Y7 receptor fragment.

8. A polynucleotide molecule according to claim 7, wherein the polynucleotide molecule comprises a nucleotide sequence showing at least 95% homology to that shown at nucleotides 1 to 1903 or nucleotides 369 to 1592 of SEQ ID NO: 4 or any portion thereof encoding a functionally equivalent NPY-Y7 receptor fragment.

9. A polynucleotide molecule according to claim 7 or 8, wherein the polynucleotide molecule comprises a nucleotide sequence substantially corresponding to that shown at nucleotides 1 to 1903 or nucleotides 369 to 1592 of SEQ ID NO: 4 or any portion thereof encoding a functionally equivalent NPY-Y7 receptor fragment.

10. A plasmid or expression vector including a polynucleotide molecule according to any one of claims 1 to 9.

11. A host cell transformed with a polynucleotide molecule according to any one of claims 1 to 9 or a plasmid or expression vector according to claim 10.

12. A host cell according to claim 11, wherein the cell is a mammalian or insect cell.

13. A host cell according to claim 12, wherein the cell is a Chinese hamster ovary (CHO) cell, human embryonic kidney (HEK) 293 cell or an insect Sf9 cell.

14. A host cell according to any one of claims 11 to 13, wherein the cell expresses the NPY-Y7 receptor or functionally equivalent fragment thereof onto the cell's surface.

15. An NPY-Y7 receptor which is characterised by the N-terminal amino acid sequence:

$MX_1X_2MX_3EKWDX_4NSSE$ (SEQ ID NO:1),

wherein X_1, X_2, X_3 and X_4 are selected from codable amino acids, or a functionally equivalent fragment of said receptor, in a substantially pure form.

16. A receptor according to claim 15, wherein said receptor is a human receptor of about 408 amino acids.

17. A receptor according to claim 16, wherein said receptor has an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

18. A receptor according to claim 15, wherein said receptor is a murine receptor of about 405 amino acids.

19. A receptor according to claim 18, wherein the receptor has an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 3.

20. An antibody or fragment thereof which specifically binds to an NPY-Y7 receptor according to any one of claims 15 to 19.

21. A non-human animal transformed with a polynucleotide molecule according to any one of claims 1 to 9 or a plasmid or expression vector according to claim 10.

22. A method for detecting agonist or antagonist agents of an NPY-Y7 receptor, comprising contacting an NPY-Y7 receptor according to any one of claims 15 to 19 or a host cell transformed according to any one of claims 11 to 14, with a test agent under conditions enabling the activation of said receptor, and detecting an increase or decrease in the receptor activity.

23. An oligonucleotide or polynucleotide probe comprising a nucleotide sequence of 10 or more nucleotides, the probe comprising a nucleotide sequence such that the probe specifically hybridises to the polynucleotide molecule according to any one of claims 1 to 9 under high stringency conditions.

24. An antisense oligonucleotide or polynucleotide molecule comprising a nucleotide sequence capable of specifically hybridising to an mRNA molecule which encodes an NPY-Y7 receptor encoded by the polynucleotide molecule according to any one of claims 1 to 9, so as to prevent translation of the mRNA molecule.

25. A method of producing NPY-Y7 receptors or functionally equivalent fragments thereof according to any one of claims 15 to 19, comprising culturing a host cell according to any one of claims 11 to 14 under conditions enabling the expression of NPY-Y7 receptors or functionally equivalent fragments thereof, and optionally recovering the receptors or functionally equivalent fragments thereof.

FIGURE 1 1/4

Human neuropeptide Y - Y7 sequence alignment

```
hy1p   1                                                  MNSTLFSQVENHSVHS  16
hy2p   1                                      MGPIGAEADENQTVEEMKVEQYGPQST  26
hy7p   1                                      -MFIMNEKWDTNSSENWHPIWNVNDT  25

hy1p  17  NFSEKNAQLLAFENDDCHLPLAMIFTLALAYGAVTI  52
hy2p  27  TPRGELVPDPEPELIDSTKLIEVQVVLILAYCSILL  62
hy7p  26  KHHLYSDINITYVNYYLHQP-QVAAIFLISLYFLIFF  60

hy1p  53  LGVSGNLALIIILKQKEMRNVTNILIVNLSFSDLL  88
hy2p  63  LGVIGNSLVIHVVIKFKSMRTVTNFFIANLAVADLL  98
hy7p  61  LCMMGNTVVCFIVMRNKHMHTVTNLFILNLAISDLL  96

hy1p  89  VAIMCLPFTFVYTLMDHWVFGEAMCKLNPFVQCVSI 124
hy2p  99  VNTLCLPFTLTYTLMGEWKMGPVLCHLVPYAQGLAV 134
hy7p  97  VGIFCMPITLLDNIIAGWPFGNTMCKISGLVQGISV 132

hy1p 125  TVSIFSLVLIAVERHQLTINPRGWRPNNRHAYVGTA 160
hy2p 135  QVSTITLTVIALDRHRCIVYHLESKISKRISFLIIG 170
hy7p 133  AASVFTLVAIAVDRFQCVVYPFKPKLTIKTAFVIM 168

hy1p 161  VIWVLAVASSLPFLIYQVMTDEPFQNVTLD---AYK 193
hy2p 171  LAWGISALLASPLAI----------FREYSLIEIIPDF 198
hy7p 169  IIWVLAITIMSPSAVMLHVQEEKYYRVRLNSQNKTS 204

hy1p 194  DKYVCFDQFPSDSHRLSYTTLLLV---LQYFGPLCF 226
hy2p 199  EIVACTEKWPGEEKSIYGTVYSLSSLLILYVLPLGI 234
hy7p 205  PVYWCREDWPNQEMRKIYTTVLFANI---YLAPLSL 237

hy1p 227  IFICYFKIYIRLKRNNM---MDKMRDNKYRSSETK 259
hy2p 235  ISFSYTRIWSKLKNHVSP--GAANDHYHQRRQKTTK 268
hy7p 238  IVIMYGRIGISLFRAAVPHTGRKNQEQWHVVSRKKQ 273

hy1p 260  RINIMLLSIVVAFAVCWLPLTIFNTVFDW--NHQII 293
hy2p 269  ----MLIVCVVVVFAVSWLPLHAFQLAVDI--DSQVL 298
hy7p 274  KIIKMLIIVALLFILSWLPLWTLMMLSDYADLSPNE 309

hy1p 294  ATCNHNLLFLLCHLTAMISTCVNPIFYGFLNKNFQR 329
hy2p 299  DLKEYKLIFTVFHIIAMCSTFANPLLYGWMNSNYRK 334
hy7p 310  LQIINIYIYPFAHWLAFGNSSVNPIIYGFFNENFRR 345

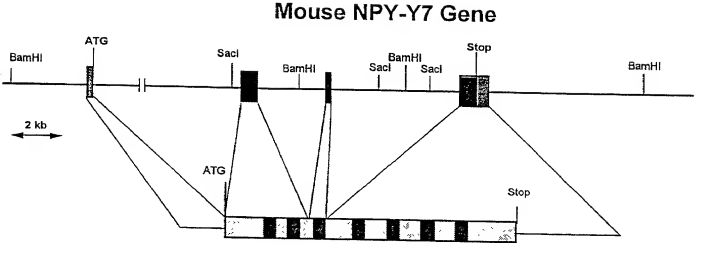
hy1p 330  DLQFFFNFCDFRSRDDDYETIAMSTMHTDVSKTSLK 365
hy2p 335  AFLSAFRCEQRLDAIHSEVSVTFKAKKNLEVRKNSG 370
hy7p 346  GFQEAFQLQLCQKRAKPMEAYTLKAKSHVLINTSNQ 381

hy1p 366  QASPVAFKKINNNDDNEKI                  384
hy2p 371  PNDSFTEATNV                          381
hy7p 382  LVQESTFQNPHGETLLYRKSAENPNRN          408
```

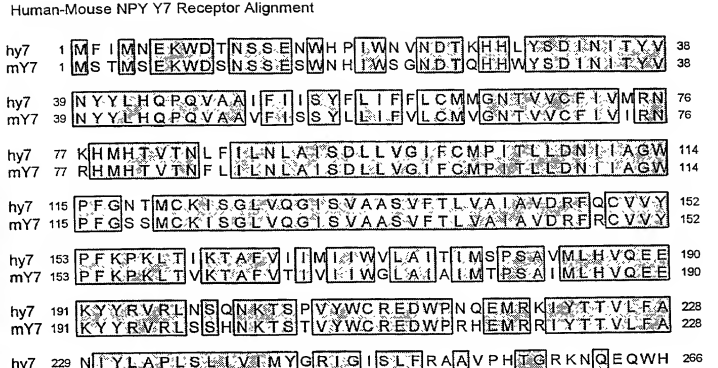
FIGURE 2

Inhibition of human [125]PYY binding with various NPY-related peptides on human Y7 membranes
[125I]PYY (50pM)
PYY
NPY
[2-36]NPY
[13-36]PYY
Leu31Pro34NPY
PP
% specific binding
100
75
50
25
0
-13
-12
-11
-10
-9
-8
-7
-6
log [AGONIST] (M)

FIGURE 3


Mouse NPY-Y7 Gene
BamHI
ATG
SacI
BamHI
BamHI
SacI
SacI
Stop
BamHI
2 kb
ATG
Stop

Human-Mouse NPY Y7 Receptor Alignment



Sequence Listings:

Applicant: Garvan Institute of Medical Research
Title of Invention: NPY-Y7 Receptor Gene

Prior Application Number: PP 4385
Prior Application Filing Date: 1998-06-29

Number of SEQ ID NOs: 5

Software: PatentIn Ver. 2.1

SEQ ID NO: 1
Length: 14
Type: PRT
Organism: Artificial Sequence

Feature:
Other Information: Description of Artificial Sequence: N-terminal consensus sequence

Sequence: 1
Met Xaa Xaa Met Xaa Glu Lys Trp Asp Xaa Asn Ser Ser Glu
1 5 10

SEQ ID NO: 2
Length: 408
Type: PRT
Organism: Homo sapiens

Sequence: 2
Met Phe Ile Met Asn Glu Lys Trp Asp Thr Asn Ser Ser Glu Asn Trp
1 5 10 15
His Pro Ile Trp Asn Val Asn Asp Thr Lys His His Leu Tyr Ser Asp
20 25 30
Ile Asn Ile Thr Tyr Val Asn Tyr Tyr Leu His Gln Pro Gln Val Ala
35 40 45
Ala Ile Phe Ile Ile Ser Tyr Phe Leu Ile Phe Phe Leu Cys Met Met

```
     50                  55                  60
Gly Asn Thr Val Val Cys Phe Ile Val Met Arg Asn Lys His Met His
 65                  70                  75                  80
Thr Val Thr Asn Leu Phe Ile Leu Asn Leu Ala Ile Ser Asp Leu Leu
                 85                  90                  95
Val Gly Ile Phe Cys Met Pro Ile Thr Leu Leu Asp Asn Ile Ile Ala
            100                 105                 110
Gly Trp Pro Phe Gly Asn Thr Met Cys Lys Ile Ser Gly Leu Val Gln
        115                 120                 125
Gly Ile Ser Val Ala Ala Ser Val Phe Thr Leu Val Ala Ile Ala Val
    130                 135                 140
Asp Arg Phe Gln Cys Val Val Tyr Pro Phe Lys Pro Lys Leu Thr Ile
145                 150                 155                 160
Lys Thr Ala Phe Val Ile Ile Met Ile Ile Trp Val Leu Ala Ile Thr
                165                 170                 175
Ile Met Ser Pro Ser Ala Val Met Leu His Val Gln Glu Glu Lys Tyr
            180                 185                 190
Tyr Arg Val Arg Leu Asn Ser Gln Asn Lys Thr Ser Pro Val Tyr Trp
        195                 200                 205
Cys Arg Glu Asp Trp Pro Asn Gln Glu Met Arg Lys Ile Tyr Thr Thr
    210                 215                 220
Val Leu Phe Ala Asn Ile Tyr Leu Ala Pro Leu Ser Leu Ile Val Ile
225                 230                 235                 240
Met Tyr Gly Arg Ile Gly Ile Ser Leu Phe Arg Ala Ala Val Pro His
                245                 250                 255
Thr Gly Arg Lys Asn Gln Glu Gln Trp His Val Val Ser Arg Lys Lys
            260                 265                 270
Gln Lys Ile Ile Lys Met Leu Leu Ile Val Ala Leu Leu Phe Ile Leu
        275                 280                 285
Ser Trp Leu Pro Leu Trp Thr Leu Met Met Leu Ser Asp Tyr Ala Asp
    290                 295                 300
Leu Ser Pro Asn Glu Leu Gln Ile Ile Asn Ile Tyr Ile Tyr Pro Phe
305                 310                 315                 320
Ala His Trp Leu Ala Phe Gly Asn Ser Ser Val Asn Pro Ile Ile Tyr
                325                 330                 335
Gly Phe Phe Asn Glu Asn Phe Arg Arg Gly Phe Gln Glu Ala Phe Gln
            340                 345                 350
Leu Gln Leu Cys Gln Lys Arg Ala Lys Pro Met Glu Ala Tyr Thr Leu
        355                 360                 365
```

```
Lys Ala Lys Ser His Val Leu Ile Asn Thr Ser Asn Gln Leu Val Gln
    370                 375                 380
Glu Ser Thr Phe Gln Asn Pro His Gly Glu Thr Leu Leu Tyr Arg Lys
385                 390                 395                 400
Ser Ala Glu Asn Pro Asn Arg Asn
                405
```

SEQ ID NO: 3
Length: 405
Type: PRT
Organism: Mus musculus

Sequence: 3

```
Met Ser Thr Met Ser Glu Lys Trp Asp Ser Asn Ser Ser Glu Ser Trp
  1               5                  10                  15
Asn His Ile Trp Ser Gly Asn Asp Thr Gln His His Trp Tyr Ser Asp
            20                  25                  30
Ile Asn Ile Thr Tyr Val Asn Tyr Tyr Leu His Gln Pro Gln Val Ala
        35                  40                  45
Ala Val Phe Ile Ser Ser Tyr Leu Leu Ile Phe Val Leu Cys Met Val
    50                  55                  60
Gly Asn Thr Val Val Cys Phe Ile Val Ile Arg Asn Arg His Met His
65                  70                  75                  80
Thr Val Thr Asn Phe Leu Ile Leu Asn Leu Ala Ile Ser Asp Leu Leu
                85                  90                  95
Val Gly Ile Phe Cys Met Pro Ile Thr Leu Leu Asp Asn Ile Ile Ala
            100                 105                 110
Gly Trp Pro Phe Gly Ser Ser Met Cys Lys Ile Ser Gly Leu Val Gln
        115                 120                 125
Gly Ile Ser Val Ala Ala Ser Val Phe Thr Leu Val Ala Ile Ala Val
    130                 135                 140
Asp Arg Phe Arg Cys Val Val Tyr Pro Phe Lys Pro Lys Leu Thr Val
145                 150                 155                 160
Lys Thr Ala Phe Val Thr Ile Val Ile Ile Trp Gly Leu Ala Ile Ala
                165                 170                 175
Ile Met Thr Pro Ser Ala Ile Met Leu His Val Gln Glu Glu Lys Tyr
            180                 185                 190
Tyr Arg Val Arg Leu Ser Ser His Asn Lys Thr Ser Thr Val Tyr Trp
```

```
        195                 200                 205
Cys Arg Glu Asp Trp Pro Arg His Glu Met Arg Arg Ile Tyr Thr Thr
    210                 215                 220
Val Leu Phe Ala Ile Ile Tyr Leu Ala Pro Leu Ser Leu Ile Val Ile
225                 230                 235                 240
Met Tyr Ala Arg Ile Gly Ala Ser Leu Phe Lys Thr Ala Ala His Cys
                245                 250                 255
Thr Gly Lys Gln Arg Pro Val Gln Cys Met Tyr Gln Glu Lys Gln Lys
            260                 265                 270
Val Ile Lys Met Leu Leu Thr Val Ala Leu Leu Phe Ile Leu Ser Trp
        275                 280                 285
Leu Pro Leu Trp Thr Leu Met Met Leu Ser Asp Tyr Thr Asp Leu Ser
    290                 295                 300
Pro Asn Lys Leu Arg Ile Ile Asn Ile Tyr Ile Tyr Pro Phe Ala His
305                 310                 315                 320
Trp Leu Ala Phe Cys Asn Ser Ser Val Asn Pro Ile Ile Tyr Gly Phe
                325                 330                 335
Phe Asn Glu Asn Phe Arg Asn Gly Phe Gln Asp Ala Phe Gln Ile Cys
            340                 345                 350
Gln Lys Lys Ala Lys Pro Gln Glu Ala Tyr Ser Leu Arg Ala Lys Arg
        355                 360                 365
Asn Ile Val Ile Asn Thr Ser Gly Leu Leu Val Gln Glu Pro Val Ser
    370                 375                 380
Gln Asn Pro Gly Gly Glu Asn Leu Gly Cys Gly Lys Ser Ala Asp Asn
385                 390                 395                 400
Pro His Arg Asn Pro
                405
```

SEQ ID NO: 4

Length: 1903

Type: DNA

Organism: Homo sapiens

Sequence: 4

```
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caggcgtgag accgcgcccg gccaatttcc tttcttagtt gcctctgccc acctcttctc 240
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gacatacaag aaacatcaaa aagattgaat gtcttaataa gagtgaagca tgtagatcag 360
tgactgctat gttcatcatg aatgagaaat gggacacaaa ctcttcagaa aactggcatc 420
ccatctggaa tgtcaatgac acaaagcatc atctgtactc agatatttaat attacctatg 480
tgaactacta tcttcaccag cctcaagtgg cagcaatctt cattatttcc tactttctga 540
tcttcttttt gtgcatgatg ggaaatactg tggtttgctt tattgtaatg aggaacaaac 600
atatgcacac agtcactaat ctcttcatct taaacctggc cataagtgat ttactagttg 660
gcatattctg catgcctata acactgctgg acaatattat agcaggatgg ccatttggaa 720
acacgatgtg caagatcagt ggattggtcc agggaatatc tgtcgcagct tcagtcttta 780
cgttagttgc aattgctgta gataggttcc agtgtgtggt ctacccttttt aaaccaaagc 840
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catctaatca gcttgtccag gaatctacat ttcaaaaccc tcatggggaa accttgcttt 1560
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tatttaaatc cattgctttt tgtggctttg cacttcaaat ttttcaaaga atgttctaaa 1740
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aagatcataa acaatcttat gttgtataaa aatacgtaga gtgacttaga catgtttgca 1860
tgaataaata tatttctaga gaacagttaa aaaaaaaaaa aaa 1903

SEQ ID NO: 5
Length: 1228
Type: DNA
Organism: Mus musculus

Sequence: 5

atgtccacca tgagcgagaa atgggactca aactcttcag aaagctggaa tcacatctgg 60
agtggcaatg atacacagca tcactggtat tcagatatca acattaccta tgtgaactac 120
tatctccacc agccccaagt ggcagctgtc ttcatcagct cctacctcct gatctttgtc 180
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tgcaagatca gtgggctggt gcaagggata tcagttgcgg cttccgtctt caccttggtt 420
gcaatagctg tggacagatt ccgctgtgtg gtctacccct ttaagccaaa gctcactgtc 480
aagacagcct ttgtcacgat tgtgatcatc tggggcctgg ccatcgccat tatgactcca 540
tctgcaataa tgttacatgt acaagaagaa aaatactacc gtgtgagact cagctcccac 600
aataaaacca gcacagtcta ctggtgtcgg gaggactggc caagacacga aatgaggagg 660
atctatacca cggtgctatt tgccatcatc tatcttgctc ctctctcact cattgttatc 720
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gaaccggtgt ctcaaaaccc aggtggggaa aatttgggat gtggaaaaag tgcagacaat 1200
ccacacagga atccttgata gaggaatg 1228

DECLARATION, POWER OF ATTORNEY AND PETITION

As a below named inventor, I hereby declare that:

My residence, post office and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought on the invention entitled:

NPY-Y7 RECEPTOR GENE

the specification of which
☐ is attached hereto ☒ was filed on **29 June 1999** as Application No. **PCT/AU99/00523** and was amended on
(if applicable).

I hereby state that I have reviewed and understand the consents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a)

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
PP 4385	Australia	29 June 1998	☒	☐
			☐	☐
			☐	☐
[Number]	**[Country]**	**[Day/Month/Year Filed]**	**Yes**	**No**

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

[Application Serial no]	**[Filing Date]**	**[Status: patented, pending, abandoned]**

I hereby declare that all statement s made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

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United States of America

with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and all future correspondence should be addressed to them.

****

Full name of sole or first inventor: Herbert HERZOG

Inventor's Signature _____ **Date:** 26/10/00

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